Supporting Online Material for

Nucleus Accumbens D2/3 Receptors Predict Trait Impulsivity and Cocaine Reinforcement

Jeffrey W. Dalley,* Tim D. Fryer, Laurent Brichard, Emma S.J. Robinson, David E. H. Theobald, Kristjan Lääne, Yolanda Peña, Emily R. Murphy, Yasmene Shah, Katrin Probst, Irina Abakumova, Franklin I. Aigbirhio, Hugh K. Richards, Young Hong, Jean-Claude Baron, Barry J. Everitt, Trevor W. Robbins

*To whom correspondence should be addressed. E-mail: jwd20@cam.ac.uk

Published 2 March 2007, Science 315, 1267 (2007)
DOI: 10.1126/science.1137073

This PDF file includes:

Materials and Methods
Figs. S1 to S5
Table S1
References
Supporting online material

Subjects: One hundred and three male Lister hooded (LH) rats, of approximately 2 months of age at the start of the study, were used. Subjects were housed in pairs in a humidity- and temperature-controlled holding room (22°C) under an alternating light/dark cycle (white lights off/red lights on from 07:30 to 19:30) in plastic floor-lined cages with free access to water. During training on the 5-choice task food was restricted to 15 g of standard laboratory chow per subject per day. On days when rats were not trained food intake was increased to 20 g per subject. Subjects destined for intravenous cocaine self-administration were housed individually after surgery and for the remainder of the experiment. All experimental procedures complied with the UK Animals (Scientific Procedures) Act of 1986 (Home Office license number PPL 80/1767).

Five-choice serial reaction time task training: Rats were trained on a 5-choice serial reaction time (5-CSRT) task using specially adapted operant chambers consisting of five, equally spaced 2.5 cm square apertures set into a curved rear wall(1). A PC using WhiskerServer software (version 2.8) and FiveChoice client (version 2.6) controlled the apparatus. Stable performance was achieved after approximately 35 daily sessions, each of which consisted of 100 discrete trials. Details of the different training stages and of the apparatus itself can be found elsewhere(2). Trials were initiated by subjects entering a food magazine located on the front wall of the chamber. After an inter-trial interval (ITI) of 5 s had elapsed, a brief light stimulus, 0.5 s in duration, was presented in a pseudo-random order in one of the five apertures. A correct response was recorded if subjects accurately tracked the spatial location of the target stimulus with a nose-poke response. A correct response was rewarded with a single food pellet (45 mg Noyes dustless pellets) delivered to the food magazine. Responses in any of the adjacent apertures (an ‘incorrect’ response) resulted in the house-light being extinguished for 5 s and the cancellation of food reward. A failure to respond within 5 s of the onset of the target stimulus was deemed an
omission and similarly resulted in a 5 s time-out period and no food delivery. Responses made during the ITI (i.e., before the onset of the target stimulus) were deemed to be premature or impulsive and led to a 5 s darkened time-out, no food reward, and a re-setting of the current trial. Performance was assessed using five variables: choice accuracy (% correct responses/correct + incorrect responses); premature responding (% premature responses/correct + incorrect + omissions); omissions (% omissions/correct + incorrect + omissions); latency to make a correct response after the onset of the target stimulus (ms); latency to collect food from the magazine after a correct trial (ms).

**Screening for high impulsivity:** Following acquisition of the 5-CSRT task (stimulus duration 0.5 s; ITI 5 s) and attainment of stable performance for at least 2 weeks, rats were screened for impulsivity for a period of 3 weeks. Each week involved rats being tested for 5 consecutive days with all but the third day consisting of the normal training parameters (i.e., 100 trials, ITI 5 s, stimulus duration 0.5 s and session duration 30 min). On the third day the ITI was increased to 7 s and the session duration increased to 45 min to allow sufficient time to complete 100 trials. Although the efficacy of this manipulation to increase premature responding diminishes with repeated exposure all rats nevertheless show a robust elevation in impulsive responding by the third exposure. The criterion for selecting impulsive rats was a level of premature responding greater than or equal to 50% on each of the long ITI sessions. The incidence of impulsivity defined in this way was typically 7%.

**Locomotor activity.** Locomotor activity was assessed on two consecutive days in 7 male LH impulsive rats and 16 male LH non-impulsive rats using 12 individual activity cages measuring 20 x 25 x 20 cm (w x h x d). Each chamber contained two photocell beams located 1 cm above the floor and spaced equally along the length of the cage. Subjects had never previously been exposed to the chambers. A ‘run’ was scored if the two beams were broken within 200 ms. Data
on each test day were collated into 40 x 3 min bins using software running on an Acorn computer and averaged across the two days of testing. Subjects were run at approximately the same time each day (12:00-16:00h) approximately 1 week after screening for impulsivity on the 5-CSRT task. The wide separation of the two photocell beams provides a precise measure of whole-body locomotor activity and is readily comparable with the measure of locomotor activity provided by the circular corridor apparatus used by Piazza and colleagues(3).

**MicroPET brain imaging:** A separate group of high impulsive (N = 6) and non-impulsive (N = 6) rats that had been prepared with an intravenous catheter but not exposed to cocaine were used for microPET brain imaging. Subjects were scanned in pairs each day; one impulsive rat and one non-impulsive rat. The order of scanning was strictly counterbalanced across day. Subjects were anaesthetized with 4% halothane vaporized in medical oxygen (1500 mL/min) and transferred to a purpose-built plastic cradle within the PET camera, which incorporated atraumatic ear bars and an incisor bar. Anesthesia was maintained at 1.5-2% during PET scanning. The temperature was monitored using a rectal thermometer throughout scanning and maintained within the normal range using a heating blanket. In addition, heart rate and blood oxygen saturation were continuously monitored by pulse oximetry. To correct for photon attenuation, a 30 min coincidence mode blank scan and a 20 min transmission scan were acquired with a rotating, $^{68}$Ge point source (~100MBq). [$^{18}$F]-Fallypride was then administered as a bolus injection (2 ml/kg) via the jugular catheter. The specific radioactivity at the time of injection was typically 60-80 GBq/µmol. The activity administered (range 2-26 MBq) was calculated from the specific activity and rat weight to produce a constant injected mass/kg of fallypride (0.47 ± 0.02 nmol/kg). There was no significant difference between the fallypride mass/kg (0.467 ± 0.023 nmol/kg versus 0.474 ± 0.018 nmol/kg), the administered activity (13.6 ± 5.6 MBq versus 12.5 ± 4.0 MBq), or the weight of the animals (440 ± 13 g versus 434 ± 12 g) for the impulsive and non-impulsive rats, respectively. Data were acquired post [$^{18}$F]-fallypride injection using a 350-650 keV energy
window and a 6 ns coincidence timing window into 52 time frames: 6 x 10 s, 3 x 20 s, 10 x 1 min, 10 x 2 min, 17 x 5 min.

\[^{18}F\]-Fallypride was prepared using a modified method of a previously described radiosynthesis (4). The precursor, (S)-2,3-dimethoxy-5-[[3-[(4-methylphenyl)-sulfonyl]oxy]-propyl]-N-[1-(2-propenyl)-2-pyrrolidinyl][methyl]-benzamide (Tosyl-Fallypride) was obtained from ABX GmbH. No-carrier-added aqueous \[^{18}F\]fluoride was produced by the \(^{18}\)O(p,n)\(^{18}\)F reaction on \(^{18}\)O-enriched water (> 97 atom %). This was transferred from the cyclotron target to the Tracerlab FXN module and separated from the \(^{18}\)O-enriched water by passage through a conditioned cartridge. \[^{18}F\]fluoride was eluted from the QMA cartridge into a Pyrex\textsuperscript{®} reaction vessel with 0.5 ml Kryptofix 2.2.2 (26mg/ml) and potassium carbonate (4.6 mg/ml) in water and acetonitrile (50/50 v/v). Using azeotropic evaporation with reduced pressure, a helium stream and heating at 95°C, the mixture was taken to dryness. Acetonitrile was twice added and evaporated to leave a residue of dry K\textsuperscript+-Kryptofix 2.2.2 -\[^{18}F\]fluoride to which a solution of the tosyl-fallypride precursor (3 mg) in acetonitrile (1 ml) was added. The solution was heated to 90°C for 15 min, cooled, and 3 ml HPLC mobile phase (acetonitrile/water/triethylamine, 56/44/0.1 v/v) added. The solution was then passed through an alumina cartridge to remove residual \[^{18}F\]fluoride, then subjected to preparative HPLC on a Luna C18 column (5 \(\mu\)m, 250 x 10 mm), eluted with acetonitrile/water/triethylamine (56/44/0.1 v/v) with UV (254 nm) and radioactivity detection. The collected \[^{18}F\]fallypride fraction was subjected to solid-phase-extraction using a cartridge, passed through a 0.22 \(\mu\)m filter to obtain \[^{18}F\]fallypride in saline with > 98% radiochemical purity. Radiochemical yields obtained at end-of-synthesis were 6.3-10.6GBq with specific radioactivities of 59-415 GBq/\(\mu\)mol.

**MRI brain template:** MR scanning of the rats scanned in the microPET was not possible due to the lack of a nearby facility. Instead, a separate group consisting of 20 adult male LH rats was
used to provide the magnetic resonance (MR) template for anatomical co-registration. This work was carried out at Nottingham University (UK) by Dr Yasmene Shah. Rats were anaesthetized with 1-2% isoflurane (70% N₂O 30% O₂) and transferred to a cradle designed to fit inside the probe of the MR system. Body temperature was maintained at 37°C via a feedback controlled heated waterbed fitted into the cradle and monitored using a rectal probe. Acquisition of images for the anatomical template was performed using a 2.35T Bruker Biospec Avance MR system. Radiofrequency pulses were transmitted using 72 mm (internal) diameter birdcage coil. An electronically decoupled receive-only coil was placed on the dorsal surface of the rat’s head. MR images were acquired using the rapid acquisition relaxation enhanced (RARE) sequence(5) with a field view of 50 mm. Anatomical volume data sets were acquired using the following imaging parameters: flip angle 90°, TE 62 ms, TR 5112.5 ms, matrix dimensions 256 x 256, averages = 32, slice width 1 mm, slice orientation coronal, number of slices 30. These imaging parameters yielded an in-plane spatial resolution of 0.2 mm x 0.2 mm and allowed coverage over the entire brain. The volume data set from each rat was registered to an anatomical volume data set of a hooded Lister rat not used in the subsequent template. This was achieved using the normalization function in SPM99 and ensured that each anatomical data set was in exactly the same space. These data sets were then averaged to give one anatomical volume data set, which served as the anatomical template. This template was subsequently re-sliced using cubic spline interpolation to cubical voxels of linear dimension 0.07 mm to aid accurate delineation of cerebellum, anterior dorsal striatum and ventral striatum regions of interest (ROI) on coronal sections using Analyze software (www.mayo.edu/bir/Software/Analyze/Analze.html).

**PET data analysis:** PET data were reconstructed using the PROMIS 3D filtered back-projection algorithm(6) into images of 180 x 180 x 151 voxels, each with dimensions of 0.5 mm x 0.5 mm x 0.5 mm. Corrections for background, randoms, dead time, normalization, attenuation and sensitivity were applied during the reconstruction process. The extreme difference in anatomical
detail between the fallypride and MR images led to poor co-registration accuracy using the automated mutual information algorithm implemented in SPM2 (www.fil.ion.ucl.ac.uk/spm).

Instead a summed fallypride image of all 52 frames was manually coregistered to the MR anatomical template using the MP4tool image fusion software (Max Planck Institute for Neurological Research, Cologne, Germany). To aid co-registration, transverse, coronal and sagittal views are simultaneously shown with this software and from a limited number of trials the estimated co-registration reproducibility was <0.2 mm in each direction. The operator performing the manual coregistration was blinded to the group status of each rat. Using the 6 rigid body parameters from the manual co-registration the fallypride images were resliced to the MR template with 7th degree B-spline in SPM2. A single bilateral cerebellum reference region (volume 86 mm³) was defined on the MR template. This region was applied to the resliced dynamic fallypride image set to generate a cerebellum time-activity curve (TAC). The resliced dynamic fallypride image set and the corresponding cerebellum TAC were inputted into the RPM software (www.cu.mrc.ac.uk/resources/rpm.shtml), which applies a voxel-by-voxel simplified reference tissue model (SRTM) using basis functions to generate a binding potential (BP) map(7).

Two striatal regions were investigated; the dorsolateral striatum and the ventral striatum, the latter included both the core and shell sub-regions of the nucleus accumbens. The goodness-of-fit for the SRTM was assessed by using RPM to fit the average of left or right striatal ROI data.

**In-vivo microdialysis:** Because binding potentials of [18F]-fallypride in the striatum may have been influenced by regional variations in DA release we used in-vivo microdialysis to measure extracellular levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in the ventral striatum of drug-naïve, impulsive (N = 5) and non-impulsive (N = 5) LH rats. Subjects were anaesthetized with halothane (see above under ‘MicroPET brain imaging’) and secured in a ‘flat-skull’ position in a stereotaxic frame. A small craniotomy was made in the skull through which a concentric-design microdialysis probe was
positioned vertically in the core sub-region of the nucleus accumbens (AP: 1.6mm; L ± 1.8mm; V: -7.5mm). The probe was perfused with artificial CSF (NaCl: 147 mM; CaCl$_2$: 1.3 mM; MgCl$_2$: 1.0 mM; KCl: 3.0 mM; sodium phosphate buffer pH 7.4) at 1 µl/min. Four 15 min basal samples were collected after a 3 h equilibration period into 2 µL aliquots of 0.2 M perchloric acid. DA, DOPAC and HVA were determined by HPLC and electrochemical detection as described previously(8). At the completion of the experiment rats were sacrificed by lethal injection of sodium pentobarbitone (200 mg/kg i.p., 1.5 ml) and perfused transcardially with normal saline followed by 4% paraformaldehyde. Coronal sections of the forebrain were stained with Cresyl-violet to allow visualization of probe tracks in the nucleus accumbens. Only subjects confirmed to have probes located in the core sub-region were included in the study.

**Intravenous catheterization:** Rats were anaesthetized with ketamine (90 mg/kg i.p) and xylazine (6.7 mg/kg, i.p) and prepared with chronic IV catheters, as described previously(9). The catheter was inserted in the right jugular vein and passed subcutaneously over the right shoulder to exit dorsally between the scapulae. Following surgery subjects were maintained in their home cage for 7 d where they were given free access to water and 20 g food/day. Catheter patency was maintained by flushing once weekly with 0.2 ml saline. Thereafter, patency was assessed on a weekly basis by testing whether blood could be pulled back from the catheter. Subjects were rejected from the study if their catheter was deemed to have failed at any stage of testing.

**Cocaine self-administration:** Following recovery from surgery subjects were re-baselined on the 5-CSRT task for 5 d, the third day again consisting of a long ITI session (i.e., 7). The acquisition of intravenous cocaine self-administration 2 d after the last 5-CSRT task session was achieved using 12 dual-lever chambers (for details see (9)). A PC using Whisker Server software (Multimedia edition; version 2.3.01) controlled the self-administration apparatus. Rats were
trained to respond under a fixed ratio schedule of reinforcement where every response on an active lever resulted in the delivery of a 0.1 ml infusion of normal saline containing 0.25 mg cocaine hydrochloride. Following each cocaine infusion the active lever was retracted for 20 s, a 2.5 cm white stimulus light above the lever was illuminated, and the house light was extinguished. After 20 s had elapsed the active lever was extended into the chamber, responding on which resulted in further infusions of cocaine. The chambers were also equipped with an inactive lever, which was neither retracted nor associated with cocaine delivery when pressed. During the first 5 d access to cocaine was restricted to 50 infusions within a time limit of 5-h. Subjects were re-tested on the 5-CSRT task 24-h after the last cocaine infusion and for six consecutive days thereafter. The ITI and stimulus duration were 5 s and 0.5 s, respectively for all test days except on the fifth test day where the ITI was increased to 7 s and the session duration lengthened to 45 min. On the tenth day of withdrawal subjects were re-introduced to the self-administration chambers for a further 5 consecutive days. This time, however, the number of cocaine infusions was increased to 150 and the sessions were increased to 8 h. Subjects were again re-tested on the 5-CSRT task 24 h after the last self-administration session and for a further six days as before. Extended access to cocaine was provided on a further two occasions; each separated by 9 days when testing on the 5-CSRT task took place.

It is important to point out the cocaine self-administration sessions were constrained by a pre-set time limit. Thus, the actual numbers of active and inactive lever responses were, for the most part, a misleading index of the rate of cocaine self-administration. The critical determinant was the time required to achieve the limit of 150 cocaine infusions, which in high impulsive rats was considerably and invariably less than the time limit of 8 hours. It should also be noted that subjects could press the active lever on more than one occasion prior to it being retracted. Thus, although the maximum number of cocaine infusions potentially achievable was limited to 150, it was nevertheless possible for subjects to make more than 150 active lever responses in a session.
**Cocaine dose-response function**

Cocaine dose-response curves were determined in a separate group of high impulsive (N = 5) and non-impulsive (N = 5) rats. Following intravenous catheterization and recovery for one week, subjects were trained to acquire intravenous cocaine self-administration (0.25 mg cocaine hydrochloride/infusion) for seven consecutive daily sessions under a continuous reinforcement schedule (FR1). During this period cocaine access was limited to a maximum of 50 infusions per 5 hour session. Thereafter, cocaine dose-response functions were generated over 5 days with two different doses of cocaine tested each day. On day 1, subject’s experienced 1000 µg cocaine under an FR1 schedule, for 60 min. The last 30 min only was used to record stable responding supported by this dose. The infusion volume was then halved (to 50 µl) to provide 500 µg cocaine per infusion. Again, only the last 30 min of responding was used for analysis. On day 2, subjects first experienced 250 µg cocaine/100 µl, for 60 min. The dose was then reduced to 125 µg by again halving the infusion volume. During subsequent days the cocaine dose was systematically reduced from 62.5 µg to 3.9 µg until, finally, the syringe was replaced with saline.

**Statistical analyses**

Behavioral variables on the 5-CSRT task were analyzed by repeated measures analysis of variance (ANOVA; SPSS type III sum-of-squares method, version 11.5) initially to compare baseline (i.e., pre-cocaine) performance between high impulsive and non-impulsive rats. Accuracy data were transformed using the formula $x' = 2\arcsin(\sqrt{x})$ prior to ANOVA. Effects on 5-CSRT task performance of extended access to intravenous cocaine self-administration were computed using 3-way ANOVA with factors group (2 levels: high impulsive; non-impulsive) cycle (4 levels: ShA’; LgA-1; LgA-2; LgA-3) and session (7 levels: 7 consecutive daily test sessions). Data generated during the cocaine self-administration study were evaluated by 3-way ANOVA of active and inactive lever responses with factors group, lever (2 levels: active;
inactive) and session (21 levels: 21 daily self-administration sessions) and by 2-way ANOVA of the rate of cocaine self-administration (mean number of cocaine infusions an hour) with factors group and session. The cocaine dose-response curves were analyzed by 2-way ANOVA with factors group and dose. Significant deviations from the requirement of homogeneity of variance were assessed by the Mauchly Sphericity test and corrected using the Huynh-Feldt (HF) epsilon ($\varepsilon$) to adjust the degrees of freedom. Significant interactions were analyzed further by ANOVA. 

$[^{18}\text{F}]$fallypride binding potentials between impulsive and non-impulsive rats were compared using ANOVA and by computing the significance of the difference between the dorsal and ventral correlation coefficients (William’s test). Microdialysis data were averaged over the four sampling intervals and analyzed by ANOVA. All tests of significance, including tests of sphericity of the covariance matrix, were performed at $\alpha = 0.05$.

Table S1. Extracellular levels of DA, DOPAC, HVA and 5-HIAA in the core sub-region of the nucleus accumbens.

<table>
<thead>
<tr>
<th></th>
<th>DA</th>
<th>DOPAC</th>
<th>HVA</th>
<th>5-HIAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-impulsive</td>
<td>10.9 ± 1.9</td>
<td>26.1 ± 3.7</td>
<td>14.2 ± 1.7</td>
<td>10.1 ± 1.9</td>
</tr>
<tr>
<td>High-impulsive</td>
<td>10.1 ± 1.0</td>
<td>21.3 ± 4.3</td>
<td>11.3 ± 1.7</td>
<td>9.34 ± 0.61</td>
</tr>
</tbody>
</table>

Values represent mean levels (± SEM) of DOPAC, HVA and 5-HIAA (each expressed as pmoles/15 min) and DA (expressed as fmoles/15 min) in drug-naïve, non-impulsive (N = 5) and high impulsive (N = 5) rats.

Fig.S1. Significant inverse relationship between 5-CSRT task impulsivity and spontaneous locomotor activity (Pearson’s R = 0.49; p = 0.021, n = 23). Data are % premature responses during a long ITI (7 s) session and total ambulatory locomotor activity averaged over two daily 2-h sessions. Data shown are the mean number of ‘runs’ recorded over 2 d of activity testing.

Fig.S2. Significant inverse relationship between impulsivity on the 5-CSRT task and D2/3 receptor availability in the ventral striatum (R=0.58; p=0.048, n=12). Data are % premature responses during the third and final long ITI (7 s) challenge session and [18F]fallypride binding potentials in the dorsal and ventral striatum. There was also a significant difference between the respective correlation coefficients derived for the dorsal and ventral striatal ROIs (t = 2.69, df 9, p < 0.05; Williams test) suggesting that the relationship between impulsivity and D2/3 receptor availability is significantly different between the two ROIs.

Fig.S3. An example of a time-activity curve fit obtained using the simplified reference tissue model (SRTM) implemented in the RPM software package. Data shown are from the ventral
striatum ROI (average of left and right striata) of a representative high impulsive subject. The cerebellum was used as the reference tissue.

**Fig. S4.** Acquisition and maintenance of intermittent, intravenous cocaine self-administration in non-impulsive (N = 8) and high impulsive (N = 8) rats. Shown are mean (± SEM) active and inactive lever responses. Access to cocaine was restricted on the first 5 d to 5 h and a maximum of 50 infusions (each containing 0.25 mg cocaine hydrochloride). Following a withdrawal period of 9 d access to cocaine was increased on each of the following 5 consecutive days to 8 h and a maximum of 150 infusions. This pattern of intermittent cocaine self-administration was repeated on two further occasions. Three-way ANOVA revealed that all subjects acquired intravenous cocaine self-administration with a significant main effect of lever (F1,14 = 1009; p < 0.001) and a significant lever x session interaction (F19,266 = 43.8; p < 0.001). There was no statistically significant difference between impulsive and non-impulsive rats in the acquisition of intravenous cocaine self-administration (group: F1,14 = 2.97; p = 0.11; group x lever: F1,14 = 1.41; p = 0.26; group x lever x session: F19,266 = 1.1; p = 0.39).

**Fig. S5.** Vertical shift in the cocaine log10 dose-response function in high impulsive rats (N = 5) compared with non-impulsive rats (N = 5). Shown are mean number of cocaine infusions during the final 30 min of responding (± SEM). The saline condition is represented by a cocaine dose of 1 µg/infusion. ANOVA revealed significant main effects of group (F1,8 = 39.20; p < 0.01) and dose (F9,72 = 53.8; p < 0.01) but no group x dose interaction (F9,72 = 1.98; εHF = 0.49; p = 0.114).
Fig.S1 Dalley et al. (supporting online material)
Fig. S2 Dalley et al. (supporting online material)
Fig.S3 Dalley et al. (supporting online material)
Fig.S4 Dalley et al. (supporting online material)
Fig. S5 Dalley et al. (supporting online material)